



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<p>(54) Title: BIOCIDAL COMPOSITION</p> <p>(57) Abstract</p> <p>A composition which comprises a cyclic thiohydroxamic acid derivative, including a metal complex thereof, together with a biocide material which is i) an agent affecting membrane permeability, ii) an isothiazolinone, or iii) an aldehyde. The cyclic thiohydroxamic acid derivative may be a zinc salt or complex of 3-hydroxy-4-methylthiazol-2(3H)-thione. The agent affecting membrane permeability may be a phenol such as o-phenylphenol or may be a biguanide, including a polymeric biguanide, or a quaternary ammonium compound. The isothiazolinone may be 1,2-benzisothiazoline-3-one or 5-chloro-2-methylisothiazolin-3-one. The aldehyde may be formaldehyde or glutaraldehyde. The composition has biocidal activity.</p>		

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BIOCIDE COMPOSITION

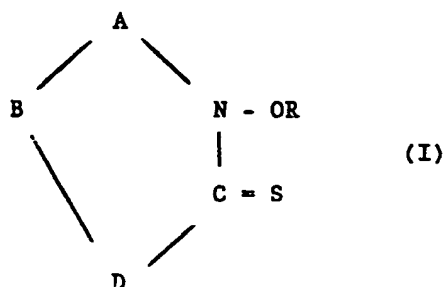
The present invention relates to compositions which are useful as industrial biocides.

Industrial biocides are useful to prevent industrial spoilage, in particular that caused by bacteria and fungi. Materials which can be used as industrial biocides have antimicrobial properties and particularly have antifungal and antibacterial properties. Such materials are useful in the preservation of paints, latices, adhesives, leather, wood, metal working fluids and cooling water.

Many compounds have been disclosed which have anti-microbial activity and some of these compounds are commercially available and are used as industrial biocides. However, whilst these materials can provide useful protection, a need remains for materials having improved characteristics.

In our European Patent Application Publication No 249328, we disclose that certain cyclic compounds have useful antimicrobial properties and can be used as biocides. We have now found that when used with other compounds having antimicrobial properties a beneficial effect can be achieved.

According to the present invention there is provided a composition which comprises A) at least one compound of the formula (I)



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or a salt or complex thereof; and

B) at least one other biologically active compound which is

- (i) an agent affecting membrane permeability;
- (ii) an isothiazolinone or an isothiazolothione; or
- (iii) an aldehyde;

wherein

A is a nitrogen or carbon atom, which may be substituted;

B and D are, independently, oxygen or sulphur or a nitrogen or carbon atom which may be substituted; or

A and/or B and/or B and/or D may be part of a ring system;

R is hydrogen, a hydrocarbyl group, a substituted hydrocarbyl group, an acyl group, a substituted acyl group or a group  $-\text{COOR}^1$ ; and

$\text{R}^1$  is a hydrocarbyl group

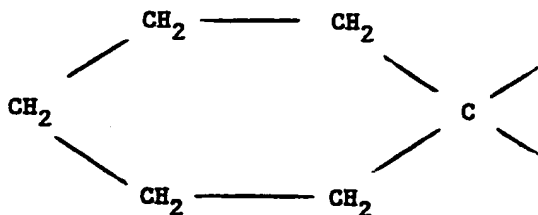
with the proviso that B and D are not both sulphur or both oxygen.

The bonds between the groups A and B and between the groups B and D may be single or double bonds but it will be appreciated that there can be only one such double bond in the ring.

The group A, and optionally one or both of groups B and D can be a group  $-\text{C}(\text{R}^2)_2-$ ; a group  $-\text{CR}^2=$ ; a group  $\text{>C}=\text{NR}^2$ ; a group  $-\text{NR}^2-$  or a group  $-\text{N}=-$ ; where  $\text{R}^2$  is a hydrogen atom, a hydrocarbyl group, a substituted hydrocarbyl group or two groups  $\text{R}^2$ , together with the carbon atom, or carbon atoms, to which they are attached form a ring.

The groups A, B and D can form part of a further ring system but generally not more than two of the groups A, B and D form part of a further ring system. The further ring system is typically a hydrocarbon ring system containing five or six carbon atoms, for example a cyclopentene, cyclohexane, cyclohexene, cyclohexadiene or benzene ring. The further ring system, if present, typically contains one or both of the groups A and B. If only the group A forms part of a ring system, this may be a cyclohexane ring of the type

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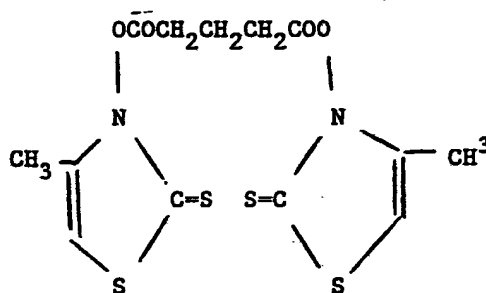
where the group A is the carbon atom with the two free valences, which are linked to the group -NOR- and B respectively. If both A and B form part of a ring system, the further ring is then fused to the azolethione ring system; for example as in 3-hydroxy-4,5,6,7-tetrahydrobenzothiazol-2(3H)-thione.

In many of the compounds used in the biocide compositions of the present invention, the groups A, B and/or D are not part of a further ring system. Thus, if A, B and/or D is a carbon atom, or substituted carbon atom, it may be, inter alia, a group -CH=, -C(CH<sub>3</sub>)=, -C(C<sub>2</sub>H<sub>5</sub>)=, -C(C<sub>6</sub>H<sub>5</sub>)=, -C(C<sub>6</sub>H<sub>4</sub>Cl)=, -C(CH<sub>3</sub>)<sub>2</sub> or C=NH. It will be appreciated that in the foregoing, the group R<sup>2</sup> is a hydrogen atom, a methyl, ethyl, phenyl or chlorophenyl group. The group typically is a hydrogen atom, a lower alkyl group, that is one containing up to five carbon atoms, an aryl group or a substituted alkyl or substituted aryl group. If the group R<sup>2</sup> is a substituted group, each substituent is a hydrocarbonoxy group, a hydrocarbonthio group, an acyl (that is a hydrocarboncarbonyl) group, an ester (that is an acyloxy) group, a halogen atom, a nitrile group or a hydroxy group.

Preferred compounds are those in which the groups A and B are both optionally substituted carbon atoms and the group D is a sulphur atom or an optionally substituted nitrogen atom. The groups A and B are preferably linked through a double bond as in the group -CR<sup>2</sup>=CR<sup>2</sup>, in which the groups R<sup>2</sup> may be the same or different. It is preferred that D is a sulphur atom.

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The group R may be a hydrogen atom, an acyl group such as benzoyl or acetyl, or an alkoxycarbonyl group such as an ethoxycarbonyl group. If the group R is a substituted group the substituent may be as disclosed for the group R<sup>2</sup> or may be a substituent which contains a further ring system of general formula I, the two ring systems being linked through the group R, for example as in the glutaryl bis ester of the formula:



Component (A) of the composition may be a salt or complex of the compound of general formula I. The salt or complex may be one which contains any metal. The metal may be a transition metal, for example a metal of group VIII, IB or IIB of the Periodic Table. Such metals include iron, copper and zinc, particularly such metals in their maximum possible valency state.

All references herein to the Periodic Table are to the Periodic Table according to Mendeleeff, as set out on the inside rear cover of "General and Inorganic Chemistry" by J.R. Partington, Second Edition published by MacMillan and Co. Limited, London.

For convenience hereafter, the compounds of the general formula I, and salts and complexes thereof will be referred to simply as "Compound I".

A wide range of Compounds I can be used as component (A) in the compositions of the present invention. The Compounds I have anti-microbial activity against a wide range of micro-organisms.

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Compounds I which can be used in the compositions of the present invention include:

3-hydroxy-4-methylthiazol-2(3H)-thione,  
3-benzoyloxy-4-methylthiazol-2(3H)-thione,  
3-hydroxy-4-phenylthiazol-2(3H)-thione,  
3-hydroxy-4,5,6,7-tetrahydrobenzothiazol-2(3H)-thione,  
3-acetoxy-4-methylthiazol-2(3H)-thione,  
the glutaryl bis-ester of 3-hydroxy-4-methylthiazol-2(3H)-thione,  
5,5-dimethyl-1-hydroxy-4-imino-3-phenylimidazolidine-2-thione,  
1-hydroxy-4-imino-3-phenyl-2-thiono-1,3-diazaspiro[4.5]decane,  
1-hydroxy-5-methyl-4-phenylimidazoline-2-thione,  
3-ethoxycarbonyloxy-4-methylthiazol-2(3H)-thione,  
4,5-dimethyl-3-hydroxythiazol-2(3H)-thione,  
4,5-dimethyl-3-acetoxythiazol-2(3H)-thione,  
4-ethyl-3-hydroxy-5-methylthiazol-2(3H)-thione,  
4-ethyl-3-acetoxy-5-methylthiazol-2(3H)-thione,  
4-(4-chlorophenyl)-3-hydroxythiazol-2(3H)-thione,  
3-hydroxy-5-methyl-4-phenylthiazol-2(3H)-thione,  
3-acetoxy-4-phenylthiazol-2(3H)-thione,

and the metal complexes and salts thereof. The metal complexes and salts thereof include ferric, cupric and zinc complexes and salts such as

the zinc complex of 3-hydroxy-4-methylthiazol-2(3H)-thione, the ferric complex of 3-hydroxy-4-methylthiazol-2(3H)-thione, the cupric complex of 1-hydroxy-4-imino-3-phenyl-2-thiono-1,3-diazaspiro[4.5]decane, the cupric complex of 4,5-dimethyl-3-hydroxythiazol-2(3H)-thione, the zinc complex of 4,5-dimethyl-3-hydroxythiazol-2(3H)-thione, and the zinc complex of 4-ethyl-3-hydroxy-5-methylthiazol-2(3H)-thione.

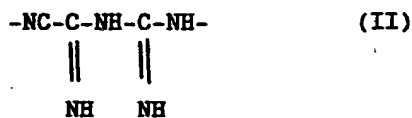
We have obtained particularly useful results when component (A) is the zinc complex or salt of 3-hydroxy-4-methylthiazol-2(3H)-thione.

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Component (B) (i) of the composition is an agent affecting membrane permeability. Action on general membrane permeability is discussed in "Pharmaceutical Microbiology" edited by W.B.Hugo and J.B.Russell, published in 1977 by Blackwell, Chapter 11, specifically at pages 202 and 204. The discussion indicates that treatment of bacterial cells with certain substances causes a leakage of a group of characteristic chemical species. Potassium ion is indicated to be the first substance to appear when such damage occurs. Materials which cause such an effect can be used as component (B) (i) of the composition of the present invention. Component (B) (i) may be a phenol and o-phenylphenol has been found to give a useful effect.

Cationic disinfectants can be effective as an agent affecting membrane permeability. By disinfectant we mean a material which is able to destroy pathogenic micro-organisms but not necessarily resistant spores as discussed on page 701 of "Medical Microbiology", 11th edition (1965) by R.Cruickshank, published by E and S Livingstone Ltd. Effective cationic disinfectants for use in the compositions of the present invention include biguanides and quaternary ammonium compounds.

Biguanides which may be used in the compositions of the present invention contain at least one biguanide unit of the formula (II):-

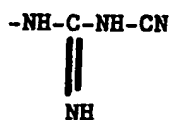


Typically the biguanide contains at least two units of the formula (II) which are linked by a bridging group which contains at least one methylene group. The bridging group may include a polymethylene chain which may optionally be interrupted by hetero atoms such as oxygen, sulphur or nitrogen. The bridging group may include one or more cyclic nuclei which may be saturated or unsaturated. It is generally preferred that the bridging group is such that there are at least three, and especially at least four,



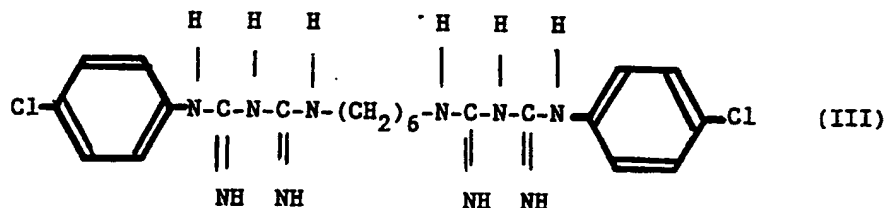
carbon atoms directly interposed between two adjacent units of the formula (II). In general it is preferred that there are not more than ten carbon atoms, especially not more than eight carbon atoms, interposed between two adjacent units of the formula (II).

The biguanide units may be terminated by any suitable group which may be a hydrocarbyl or substituted hydrocarbyl group or which may be an amine group or an amine hydrochloride group or by a group



If the terminating group is a hydrocarbyl group this may be an alkyl, cycloalkyl or aryl group or may be an alkyl, cycloalkyl or aryl group or may be a combination thereof as in an aralkyl group. If the terminating group is a substituted hydrocarbyl group, the substituent can be any substituent which does not have an undesirable adverse effect on the microbial activity of the biguanide compound and typically is a hydrocarbonoxy group, a hydrocarboncarbonyl (that is an acyl) group, an ester (that is an acyloxy) group, a halogen atom or a nitrile group and there may be more than one substituent, for example more than one halogen atom.

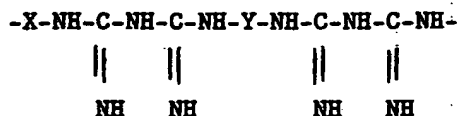
A suitable biguanide is a material which contains two units of the formula (II) and in which the units are linked by a polymethylene group, particularly a hexamethylene group. The terminating groups may be 4-chlorophenyl groups, for example as in the compound of formula (III):-



The compound of formula (II) is available as a chlorhexidine salt.

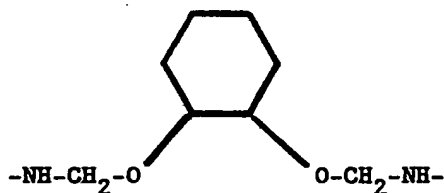
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The biguanide may alternatively be a polymeric biguanide, for example a linear polymeric biguanide which has a recurring polymer unit represented by the formula



wherein X and Y may be the same or different and represent bridging groups in which together the total number of carbon atoms directly interposed between the pairs of nitrogen atoms linked by X and Y is at least 9 and not more than 17.

The bridging groups X and Y may consist of polymethylene chains, optionally interrupted by hetero atoms, for example, oxygen, sulphur or nitrogen. X and Y may also incorporate cyclic nuclei which may be saturated or unsaturated, in which case the number of carbon atoms directly interposed between the pairs of nitrogen atoms linked by X and Y is taken as including that segment of the cyclic group, or groups, which is the shortest. Thus, the number of carbon atoms directly interposed between the nitrogen atoms in the group

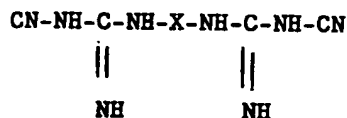


is 4 and not 8.

The preferred polymeric biguanide for use in the present invention is poly(hexamethylene biguanide), in which X and Y both represent the  $-(CH_2)_6-$  group.

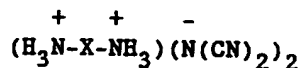
Polymeric biguanides may be prepared by the reaction of a bisdicyanidiamide having the formula

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with a diamine  $\text{H}_2\text{N-Y-NH}_2$ , wherein X and Y have the meanings defined previously herein;

or by reaction between a diamine salt of dicyanamide having the formula



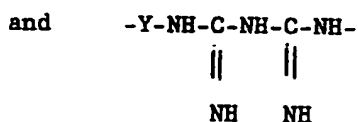
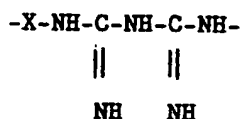
with a diamine  $\text{H}_2\text{N-Y-NH}_2$  wherein X and Y have the meanings defined previously herein. These methods of preparation are described in UK Patent Specifications Nos. 702,268 and 1152243 respectively, and any of the polymeric biguanides described therein may be used as component (B)(i) in the composition of the present invention.

The polymer chains are terminated either by an amino hydrochloride group or by an  $\text{-NH-C-NH-CN}$  group, and the terminating



groups may be the same or different on each polymer chain.

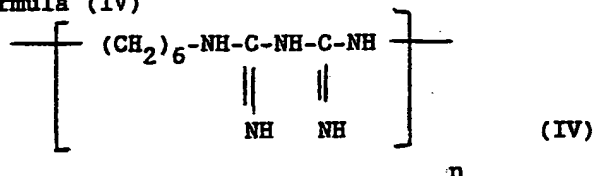
Typically, the polymeric biguanides are obtained as mixtures of polymers in which the polymer chains are of different lengths, the number of individual biguanide units, i.e.



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together being from 3 to about 80.

In the case of the preferred poly(hexamethylene biguanide) having the formula (IV)



the value of  $n$  is in the range from 6 to 15, the average molecular weight of the polymer mixture being from about 1100 to about 3300.

The biguanides are used as salts with suitable inorganic or organic acids, for example as the hydrochloride salts or the acetate or gluconate.

The component (B)(i) may be a quaternary ammonium salt of the general formula (V):-



where

$\text{R}^3$  is a hydrocarbyl group or substituted hydrocarbyl group;  
 $\text{R}^4$ ,  $\text{R}^5$  and  $\text{R}^6$  may be the same or different and are a hydrogen atom or a hydrocarbyl group or a substituted hydrocarbyl group; and

$\text{E}$  is an anion having a valency and being in an amount to give a neutral compound;

wherein

$\text{R}^3$ ,  $\text{R}^4$ ,  $\text{R}^5$  and  $\text{R}^6$  together contain at least six aliphatic carbon atoms or two or more of  $\text{R}^4$ ,  $\text{R}^5$  and  $\text{R}^6$ , together with the nitrogen atom to which they are attached, form one or more heterocyclic rings.

Preferred quaternary ammonium salts are those in which  $\text{R}^3$  is a group containing at least six aliphatic carbon atoms. If any of the groups  $\text{R}^3$ ,  $\text{R}^4$ ,  $\text{R}^5$  or  $\text{R}^6$  is a substituted hydrocarbyl group the substituent may be a hydrocarboxy group, an acyl group, an acyloxy group, a nitrile group, a hydroxy group or a halogen atom.

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The hydrocarbonyl group may be an alkyl, cycloalkyl or aryl group or a mixture of such groups as in an alkaryl group. The quaternary ammonium compound may be one in which not more than two of the groups  $R^3$ ,  $R^4$ ,  $R^5$  and  $R^6$  are alkyl groups containing at least six carbon atoms. In a compound of the foregoing type the remaining groups are preferably hydrogen, lower alkyl (as previously defined herein) or a group containing an aromatic ring such as a benzyl group. Alternatively, the residual groups may form a heterocyclic ring, for example a pyridine ring or a nitrogen containing adamantane structure.

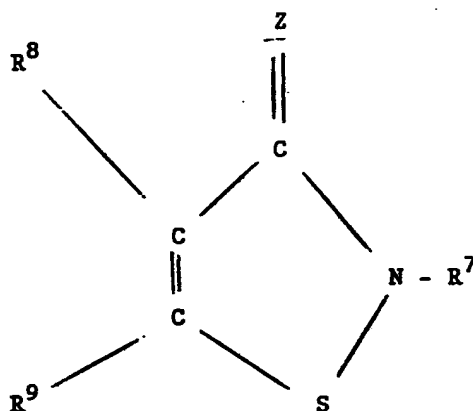
The anion E is preferably a mono-valent anion such as a halide anion, a bisulphate ( $\text{HSO}_4$ ) anion or an alkylsulphate anion.

Quaternary ammonium compounds which may be used as component (B)(i) in the composition of the present invention include diethyldodecylbenzyl ammonium chloride; dimethyloctadecyl-(dimethylbenzyl)ammonium chloride; dimethyldidecylammonium chloride; dimethyldidodecylammonium chloride; trimethyl-tetradecylammonium chloride; benzyldimethyl( $\text{C}_{12}$ - $\text{C}_{18}$  alkyl)ammonium chloride; dichlorobenzyldimethyldodecylammonium chloride; hexadecylpyridinium chloride; hexadecylpyridinium bromide; hexadecyltrimethylammonium bromide; dodecylpyridinium chloride; dodecylpyridinium bisulphate; benzyldodecyl-bis(beta-hydroxyethyl)ammonium chloride; dodecylbenzyltrimethylammonium chloride; benzyldimethyl( $\text{C}_{12}$ - $\text{C}_{18}$  alkyl) ammonium chloride; benzyldimethyl ( $\text{C}_{12}$ - $\text{C}_{16}$  alkyl) ammonium chloride; trimethyl ( $\text{C}_{12}$ - $\text{C}_{16}$  alkyl) ammonium bromide; dodecyldimethylethyl ammonium ethylsulphate; dodecyldimethyl-(1-naphthylmethyl)ammonium chloride; hexadecyldimethylbenzyl ammonium chloride; dodecyldimethylbenzyl ammonium chloride and 1-(3-chloroallyl)-3,5,7-triaza-1-azonia-adamantane chloride. The quaternary ammonium salt may be benzyldimethyl( $\text{C}_{12}$ - $\text{C}_{16}$ )alkyl ammonium chloride.

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Component (B)(ii) of the composition is an isothiazolinone or an isothiazolothione.

The isothiazolinone or isothiazolothione derivative which is component (B)(ii) of the composition is typically a compound of the general formula:



wherein:

- Z is an oxygen or sulphur atom;
- R<sup>7</sup> is a hydrogen atom, a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted hydrocarbylthio group, a substituted or unsubstituted hydrocarbyloxy group, a carbamoyl group or a cation;
- R<sup>8</sup> is a hydrogen atom, a halogen atom, a cyano group, or a substituted or unsubstituted hydrocarbyl group;
- R<sup>9</sup> is a hydrogen atom, a halogen atom, a cyano group, or a substituted or unsubstituted hydrocarbyl group; or
- R<sup>8</sup> and R<sup>9</sup>, together with the carbon atoms to which they are attached, form a five- or six-membered ring, which may optionally be substituted.

Preferably component (B)(ii) is at least one isothiazolinone derivative, that is a compound in which Z is an oxygen atom. If the groups  $R^7$ ,  $R^8$  and  $R^9$  are, or contain, substituted hydrocarbyl groups, the substituents may be as disclosed for the group  $R^2$  and are typically halogen, alkoxy or alkylthio, particularly those in which the alkyl groups contain 1 to 4 carbon atoms. If  $R^7$  is a carbamoyl group, this of the general type  $-\text{CONHR}^{10}$  where  $R^{10}$  is a hydrogen atom or a hydrocarbyl group, which may be substituted. It is generally preferred that the group  $R^7$  is a hydrogen atom or a lower alkyl group (as previously defined herein).  $R^7$  is especially hydrogen or a methyl group.

$R^8$  and  $R^9$  may, together with the carbon atoms to which they are attached, form a five- or six-membered ring, which may be substituted, the substituents typically being halogen, alkyl, alkoxy or alkylthio groups. The ring thus obtained may contain a heteroatom, for example a nitrogen atom but in general  $R^8$  and  $R^9$  form a hydrocarbon ring such as a benzene, cyclopentene or cyclohexene ring. Alternatively,  $R^8$  and  $R^9$  are separate groups and one or both of  $R^8$  and  $R^9$  can be a hydrogen atom. It is generally preferred that at least one of  $R^8$  and  $R^9$  is other than a hydrogen atom and is, particularly, a halogen atom, for example chlorine or a lower alkyl group.

Isothiazolinone compound (Z is oxygen) which can be used as component (B)(ii) of the mixture include 2-methylisothiazolin-3-one ( $R^7$  is methyl,  $R^8$  and  $R^9$  are both hydrogen); 5-chloro-2-methylisothiazolin-3-one ( $R^7$  is methyl,  $R^8$  is hydrogen and  $R^9$  is chlorine); mixtures of the foregoing two compounds; 4,5-dichloro-2-methylisothiazolin-3-one ( $R^7$  is methyl and  $R^8$  and  $R^9$  are both chlorine); 1,2-benzisothiazolin-3-one ( $R^7$  is hydrogen and  $R^8$  and  $R^9$ , together with the carbon atoms to which they are attached, form a benzene ring); 4,5-trimethylene-4-isothiazolin-3-one ( $R^7$  is hydrogen and  $R^8$  and  $R^9$  together with the carbon atoms to which they are attached, form a cyclopentene ring); and 2-methyl-4,5-trimethylene-4-isothiazolin-3-one ( $R^7$  is methyl and  $R^8$  and  $R^9$  together with the carbon atoms to which they are attached, form a cyclopentene ring).

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In component (B)(ii), if  $R^7$  is a cation this may be a cation having a valency of more than one but is particularly a monovalent cation such as an alkali metal, an amine or quaternary ammonium cation.

Component (B)(iii) of the composition is an aldehyde. Suitable aldehydes are formaldehyde and glutaraldehyde.

Useful results have been achieved when component (B) is o-phenylphenol, chlorhexidine hydrochloride salt, poly(hexamethylene biguanide) hydrochloride salt,  $(C_{12}-C_{16})$  alkyl dimethyl benzyl ammonium chloride, a mixture of quaternary ammonium chlorides including  $(C_{12}-C_{16})$  alkyl dimethyl benzyl, octyldecyl dimethyl, dioctyl dimethyl and didecyl dimethyl ammonium chloride, 5-chloro-2-methylisothiazolin-3-one, 1,2-benzisothiazolin-3-one, 2-methyl-4,5-trimethylene-4-isothiazolin-3-one, formaldehyde or glutaraldehyde.

Useful compositions in accordance with the present invention comprise

- A) the zinc complex or salt of 3-hydroxy-4-methylthiazol-2(3H)-thione; and
- B) o-phenylphenol, chlorhexidine hydrochloride salt, poly(hexamethylene biguanide) hydrochloride salt,  $(C_{12}-C_{16})$  alkyl dimethyl benzyl ammonium chloride, a mixture of quaternary ammonium chlorides including  $(C_{12}-C_{16})$  alkyl dimethyl benzyl, octyldecyl dimethyl, dioctyl dimethyl and didecyl dimethyl ammonium chloride, 5-chloro-2-methylisothiazolin-3-one; 1,2-benzisothiazolin-3-one; 2-methyl-4,5-trimethylene-4-isothiazolin-3-one, formaldehyde or glutaraldehyde.

The relative proportions of components (A) and (B) are dependent on the particular components, especially component (B). The antimicrobial activity of materials which can be used in the compositions of the present invention varies quite widely, this being particularly so in respect of materials which can be used as component (B), some of which are effective at a concentration of less



than 10 ppm by weight whilst other materials may be used in amounts as high as 0.1% by weight to achieve an effect. Hence, depending on the particular combination of (A) and (B), the relative proportions thereof, by weight, may be from 100:1 to 1:50.

The composition of the present invention may be prepared by mixing components (A) and (B) together using any suitable technique depending on the physical state of the components and their solubility. Thus, A and B may both be solids and be mixed together using any suitable solids mixing technique. Alternatively, A and B may be both be liquids and be mutually miscible. A and B may both be soluble in the same solvent and be mixed together as solutions in said solvent. A further alternative is to suspend one component which is a solid in a liquid medium which is the other component or is a solution of the other component in a liquid medium in which only one of A and B is soluble.

The mixtures of the present invention provide improved anti-microbial activity allowing lower concentrations of component (A) to be used.

The compositions of the present invention provide good wet state preservation making the compositions advantageous for use as a cutting fluid preservative and also in cooling water applications. Wood and leather preservation is another advantageous field of application of the compositions. The compositions of the present invention can also be incorporated into paint, as paint film fungicide and many of the compositions can be used without the addition of a further active component.

The compositions of the present invention may consist only of components (A) and (B). However, typically the composition comprises the components (A) and (B) as a solution, suspension or emulsion in a suitable liquid medium such as water. The composition may comprise a suspension or emulsion of the components (A) and (B), or a solution thereof, in a liquid medium in which one, or both, of the components is insoluble.

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The composition may be incorporated into a medium to be protected against micro-organisms using any suitable mixing technique. The composition is incorporated into the medium to be protected in an amount to provide from 0.00002 to 5% by weight of the composition of (A) and (B) relative to the total composition, more preferably from 0.00005 to 1% by weight of (A) and (B). It will be appreciated that the quantity of the composition of (A) and (B) required will be dependent on various factors such as the medium to be protected, the micro-organisms against which protection is desired and the extent of protection required.

If the composition is being used to preserve a solid substrate such as leather or wood, the composition may be applied directly to the substrate or may be incorporated into a coating composition such as a paint, varnish or lacquer which is then applied to the substrate. Alternatively, the solid material may be impregnated with the composition of the present invention.

The compositions of the present invention can be used for the treatment of various media to inhibit the growth of micro-organisms.

Thus, as a further aspect of the present invention there is provided a method for inhibiting the growth of micro-organisms on, or in, a medium which comprises treating the medium with a composition of (A) and (B) as hereinbefore defined.

The composition can be used in conditions in which micro-organisms grow and cause problems such as, for example, in aqueous environments including cooling water systems, paper mill liquors, metal working fluids, geological drilling lubricants, polymer emulsions, and emulsion paints. The composition can also be used to impregnate solid materials such as wood or leather or can be coated onto the surfaces thereof directly or incorporated into a paint, varnish or lacquer.

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As a further aspect the present invention provides a material which is susceptible to attack by micro-organisms and which contains a composition of (A) and (B) in an amount sufficient to at least reduce attack by micro-organisms.

Further aspects of the present invention are described in the following illustrative examples.

In the following examples, the products obtained were subjected to microbiocidal evaluation. The microbiological testing was effected, as follows:

#### Preparation of Soil Organism Sample

Soil was taken from a depth of up to 100 mm, the top 25 mm being discarded. To each gramme of soil (wet weight) was added 2 cm<sup>3</sup> of sterile saline (0.85% w/v sodium chloride). The resulting mixture was stirred for 30 minutes and allowed to settle for five minutes. The liquid was separated from large particulate matter using a syphon.

20 cm<sup>3</sup> of the resulting liquid was added to malt broth to give a final volume of 1 dm<sup>3</sup>.

#### Evaluation of Microbial Activity

10 cm<sup>3</sup> of the soil/broth mixture prepared as described were placed into 25 cm<sup>3</sup> bottles. To the contents of each bottle were added quantities of one or more biocides to give an 11 x 11 array containing different relative proportions of biocide or biocide mixture. The bottles were vortex mixed for 1-3 seconds and incubated for one week at 25°C without agitation.

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The bottles were inspected and any containing visible bacterial or fungal growth were recorded and the contents thereof discarded. 10 mm<sup>3</sup> aliquot samples were removed from the remaining bottles and these were placed on malt agar plates, allowed to dry and incubated at 25°C for two to three days. The presence or absence of growth was recorded and the bottles providing the aliquots giving growth were discarded.

Immediately after removing the 10 mm<sup>3</sup> aliquot samples, 200 mm<sup>3</sup> portions of freshly prepared Soil Organism Sample were added to the remaining bottles. After a further week, 10 mm<sup>3</sup> aliquot samples were removed, placed on malt agar plates and evaluated for growth.

The procedure described was continued until 3 portions, each of 200 mm<sup>3</sup>, of freshly prepared Soil Organism Sample had been added to the bottles containing the initial soil/broth mixture.

#### Examples 1 to 10

A number of compounds and mixtures were tested in the manner described. The concentrations of biocide, or biocide mixture, which prevented any viable organisms giving rise to growth in the fourth 10 mm<sup>3</sup> aliquot placed on a malt agar plate were noted as providing effective control of the soil organisms.

The results obtained were used to plot the variation of FLC with the relative proportions of the biocide components. These results are set out in accompanying Figures 1 to 11, wherein

Figure 1 is a plot of the variation of FLC using ZHMT (the zinc complex or salt of 3-hydroxy-4-methylthiazol-2(3H)-thione) or 1,2-benzisothiazolin-3-one (BIT) or mixtures thereof;

Figure 2 is a plot of the variation of FLC using ZHMT or formaldehyde or mixtures thereof;

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Figure 3 is a plot of the variation of FLC using ZHMT or 1,2-dibromo-2,4-dicyanatobutane (T38) or mixtures thereof;

Figure 4 is a plot of the variation of FLC using ZHMT or glutaraldehyde or mixtures thereof;

Figure 5 is a plot of the variation of FLC using ZHMT or chlorhexidine or mixtures thereof;

Figure 6 is a plot of the variation of FLC using ZHMT or 2-methyl-4,5-trimethylene-4-isothiazolin-3-one (MTI) or mixtures thereof;

Figure 7 is a plot of the variation of FLC using ZHMT or 5-chloro-2-methylisothiazolin-3-one (CIT) or mixtures thereof;

Figure 8 is a plot of the variation of FLC using ZHMT or (C<sub>12</sub>-C<sub>16</sub>)alkyldimethyl-benzylammonium chloride (VCL) or mixtures thereof;

Figure 9 is a plot of the variation of FLC using ZHMT or o-phenylphenol or mixtures thereof;

Figure 10 is a plot of the variation of FLC using ZHMT or a mixture of quaternary ammonium chlorides (available as Bardac 205M (205) from Lonza Inc) or mixtures thereof;

Figure 11 is a plot of the variation of FLC using ZHMT or a poly(hexamethylenebiguanide) hydrochloride having an average molecular weight in the range 1850 to 3300 (PHB) or mixtures of ZHMT and PHB.

In Figures 1 to 11, the concentration of the biocides are given as a proportion of the fractional lethal concentration (FLC) where

$$\text{FLC} = \frac{\text{Concentration of compound in mixture which controls growth}}{\text{Concentration of compound alone which controls growth.}}$$

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In Figures 1 to 11, the dotted straight line represents the relationship if an additive effect is achieved. The solid line represents the relationship as determined experimentally. In Figure 3, using a mixture which is not in accordance with the present invention, the dotted and solid lines coincide showing that this mixture gives an additive effect.

It should be noted that not all of the biocides used with ZHMT were effective under the test conditions described even at the highest concentration tested and with these materials a value of one on the axis of the graph represents a numerical extrapolation to a point where the compound alone gives control.

From the plots in Figures 1 to 11, it is possible to determine the minimum value of the FLC of the mixtures used and the proportions of the two components which are required to attain this minimum value. This information is set out in the following Table together with results using further mixtures which are not represented in Figures 1 to 11 including mixtures, not in accordance with the present invention, which did not give a minimum value of FLC.

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TABLE

Example or Comp Ex.	ZHMT (a) Amount (ppm) (b)	Biocide		Total FLC (d)
		Type (c)	Amount	
		(c)	(ppm) (b)	
A	50	NIL	0	1.0
B	0	BIT	63	1.0
1	20	BIT	22	0.75
C	0	FA	1600	1.0
2	12.5	FA	400	0.5
D	12.5	GA	2000	1.0+
3	15	GA	1200	0.75+
E	0.0	CH	6000	1.0
4	12.5	CH	1200	0.45
F	12.5	MTI	60.0	1.0+
5	17.5	MTI	36	0.8+
G	0.0	CIT	2.4	1.0
6	17.5	CIT	1.2	0.85
H	0.0	VCL	350	1.0
7	15	VCL	140	0.7
I	0.0	OPP	300	1.0
8	20.0	OPP	105	0.75
J	0	205	125	1.0
9	12.5	205	37.5	0.55
K	12.5	PHB	200	1.0+
10	15	PHB	120	0.75+
L	0	T38	100	1.0++
M	30	T38	70	1.3++
N	40	T38	10	0.9++
O	0.0	BRO	49.2	1.0
P	62.5	BRO	49.2	2.25
Q	75	BRO	24.6	2.0
R	0	DS	1000	1++
S	62.5	DS	1000	2.25++
T	75	DS	500	2.0++
U	0	DA	1000	1.0++
V	87.5	DA	800	2.55++

Notes to Table

- (a) ZHMT is the zinc complex or salt of  
3-hydroxy-4-methylthiazol-2(3H)-thione and can be prepared  
as described in Example 2 of EP-A-249328.
- (b) Amount is the concentration of the active component in  
parts per million w/v of the liquid mixture.
- (c) BIT is 1,2-benzisothiazoline-3-one  
FA is formaldehyde  
GA is glutaraldehyde  
CH is chlorhexidine  
MTI is 2-methyl-4,5-trimethylene-4-isothiazolin-3-one  
CIT is 5-chloro-2-methylisothiazolin-3-one  
VCL is (C<sub>12</sub>-C<sub>16</sub>)alkyldimethylbenzyl ammonium chloride  
OPP is o-phenylphenol  
205 is a mixture of quaternary ammonium chlorides available  
as Bardac 205M from Lonza Inc.  
PHB is poly(hexamethylene biguanide)hydrochloride having an  
average molecular weight in the range 1850 to 3300  
T38 is 1,2-dibromo-2,4-dicyanatobutane  
BRO is 5-bromo-5-nitro-1,3-dioxan  
DS is 2,3,5,6-tetrachloro-4-methylsulphonylpyridine  
DA is  
3-(2-chlorobenzyl)-2,2-dimethyl-4-(triazol-1-yl)butan-3-ol
- (d) FLC is the fractional lethal concentration and is the  
concentration which prevents growth in the "Evaluation of  
microbial activity" test as described herein and for any  
compound is given by the relationship:-

FLC = Concentration of compound in mixture which prevents growth  
Concentration of compound alone which prevents growth



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- + At the maximum level used, these compounds alone did not prevent growth under the test conditions described and were effective to prevent growth only in combination with a quantity of ZHMT. With these compounds, FLC is calculated as follows:-

$$\frac{(\text{Concn. of compound in mixture preventing growth})}{\text{Highest concn of compound used}} \times [1 - (\text{FLC of ZHMT at highest concn of other compound})]$$

Hence, if the highest concentration of a compound which prevents growth is 1000 ppm in the presence of 10 ppm of ZHMT, the relationship is

$$\frac{(\text{Concn of compound in mixture preventing growth})}{1000} \times \frac{(1-10)}{50}$$

that is 0.8  $\frac{(\text{Concentration of compound in mixture prevent growth})}{1000}$

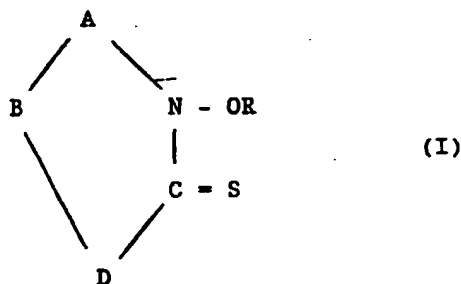
- ++ At the maximum level used, these compound alone did not prevent growth under the test conditions described and were effective to prevent growth only in combination with a quantity of ZHMT but did not give a minimum value of FLC. With these compounds, FLC is determined using the highest concentration of the particular compound used, for example a compound alone does not give control at 1600 ppm but in a mixture with ZHMT control is achieved with 40 ppm of ZHMT and 1200 ppm of the compound, the FLC for this mixture is

$$\frac{40}{50} + \frac{1200}{1600} = 0.8 + 0.75 = 1.55$$

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CLAIMS

1. A composition which comprises  
 (A) at least one compound of the formula (I)



or a salt or complex thereof; and

- (B) at least one other biologically active compound which is  
 (i) an agent effecting membrane permeability;  
 (ii) an isothiazolinone or an isothiazolothione; or  
 (iii) an aldehyde;.

wherein

- A is a nitrogen or carbon atom, which may be substituted;  
 B and D are, independently, oxygen or sulphur or a nitrogen or carbon atom which may be substituted; or  
 A and/or B and/or D may be part of a ring system;  
 R is hydrogen, a hydrocarbyl group, a substituted hydrocarbyl group, an acyl group, a substituted acyl group or a group  $-COOR^1$ ; and  
 $R^1$  is a hydrocarbyl group with the proviso that B and D are not both sulphur or both oxygen.

2. A composition as claimed in claim 1 wherein component (A) is a compound in which A, and optionally one or both of the groups B and D are a group  $-C(R^2)_2-$ , a group  $-CR^2=$ , a group  $C=NR^2$ , a group  $-NR^2$  or a group  $-N=$ , wherein

- $R^2$  is a hydrogen atom or an alkyl group containing up to five carbon atoms

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3. A composition as claimed in either claim 1 or claim 2 wherein component (A) is the zinc salt or complex of 3-hydroxy-4-methylthiazol-2(3H)-thione.

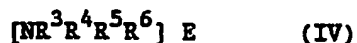
4. A composition as claimed in any one of claims 1 to 3 wherein component (B)(i) is a phenol.

5. A composition as claimed in any one of claims 1 to 3 wherein component (B)(i) is a cationic disinfectant.

6. A composition as claimed in claim 5 wherein component (B)(i) is a biguanide or a quaternary ammonium compound.

7. A composition as claimed in claim 6 wherein component (B)(i) is a chlorhexidine salt or a poly(hexamethylene biguanide) having an average molecular weight of from about 1100 to about 3300.

8. A composition as claimed in claim 6 wherein component (B)(i) is a quaternary ammonium salt of the general formula (IV)



where:

$\text{R}^3$  is a hydrocarbyl group or substituted hydrocarbyl group;  
 $\text{R}^4$ ,  $\text{R}^5$  and  $\text{R}^6$  may be the same or different and are a hydrogen atom, a hydrocarbyl group or a substituted hydrocarbyl group; and  
 $\text{E}$  is an anion having a valency, and being in an amount, to give a neutral compound;

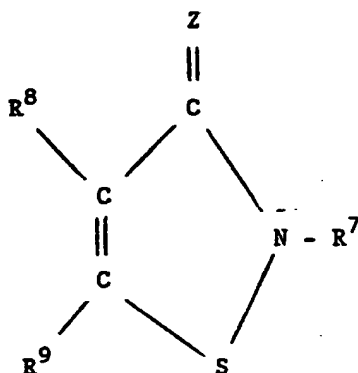
wherein

$\text{R}^3$ ,  $\text{R}^4$ ,  $\text{R}^5$  and  $\text{R}^6$  together contain at least six aliphatic carbon atoms or two or more of  $\text{R}^4$ ,  $\text{R}^5$  and  $\text{R}^6$ , together with the nitrogen atom to which they are attached, form one or more heterocyclic rings.

9. A composition as claimed in claim 8 wherein component (B)(i) is benzyl dimethyl ( $\text{C}_{12}$ - $\text{C}_{16}$ ) alkyl ammonium chloride.

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10. A composition as claimed in either claim 1 or claim 2 wherein component (B)(ii) is a compound of the general formula



wherein

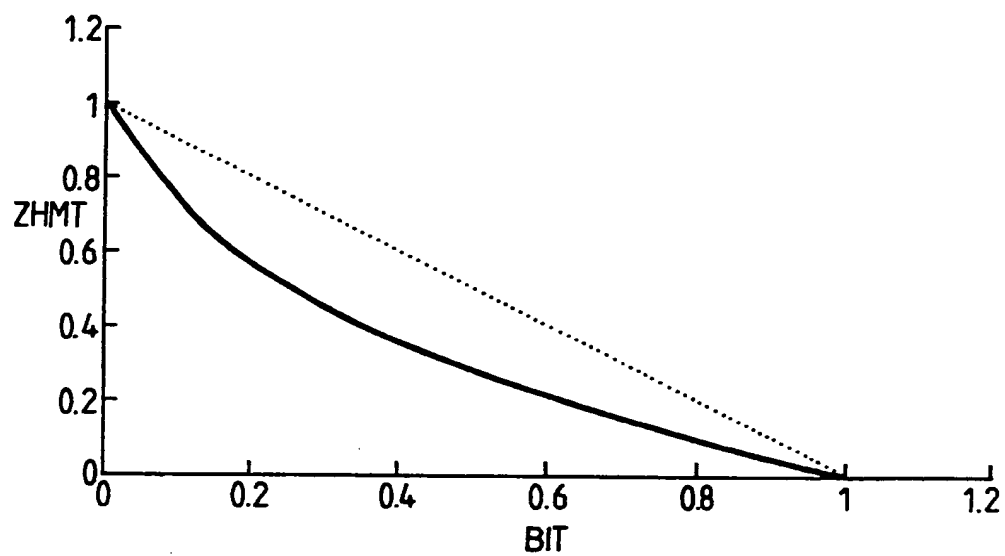
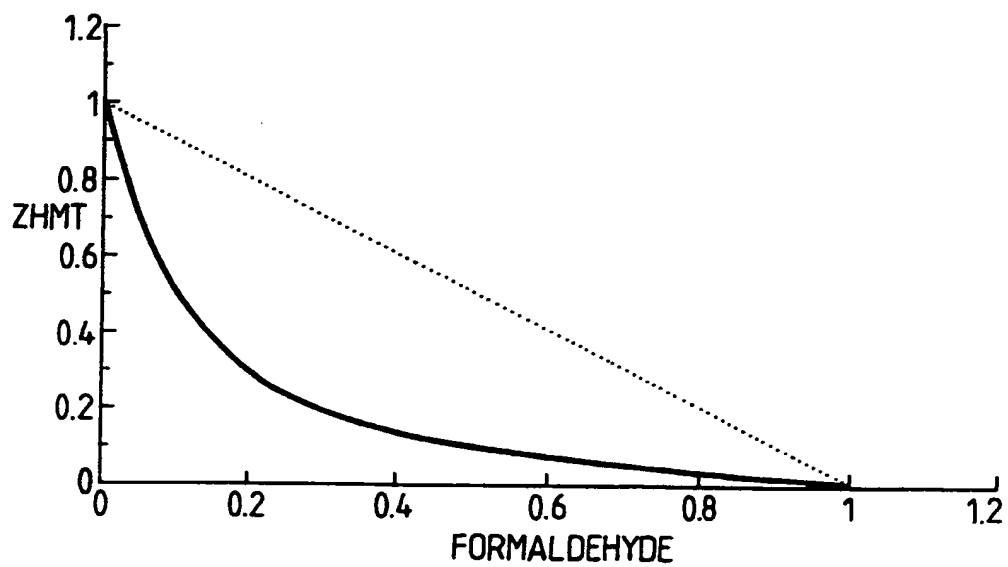
- Z is an oxygen or sulphur atom;
- R<sup>7</sup> is a hydrogen atom, a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted hydrocarbylthio group, a substituted or unsubstituted hydrocarbyloxy group, a carbamoyl group or a cation;
- R<sup>8</sup> is a hydrogen atom, a halogen atom, a cyano group or a substituted or unsubstituted hydrocarbyl group;
- R<sup>9</sup> is a hydrogen atom, a halogen atom, a cyano group, or a substituted or unsubstituted hydrocarbyl group; or
- R<sup>8</sup> and R<sup>9</sup> together with the carbon atoms to which they are attached form a five- or six- membered ring, which may optionally be substituted.

11. A composition as claimed in claim 10 wherein component (B)(ii) is a compound in which Z is an oxygen atom and R<sup>7</sup> is a hydrogen atom or an alkyl group containing up to five carbon atoms.

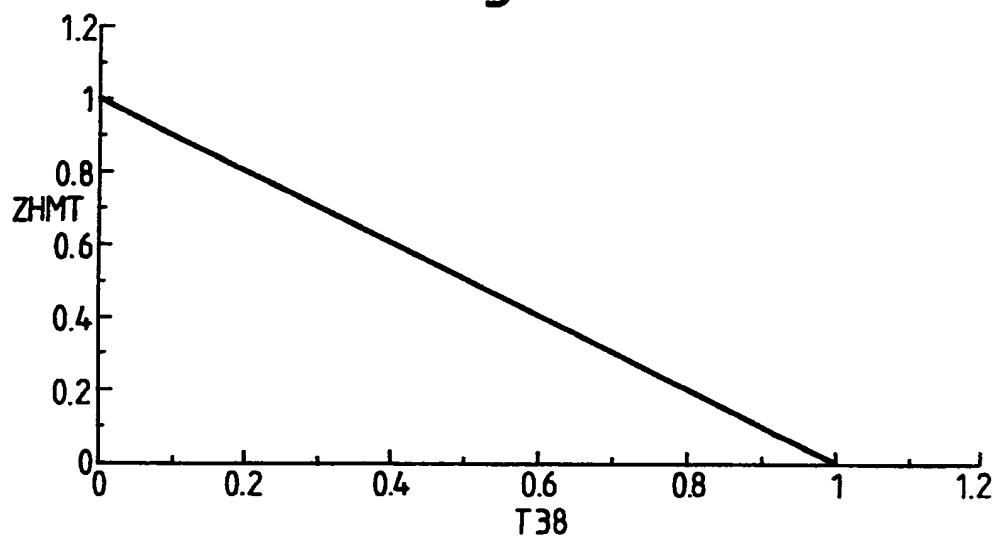
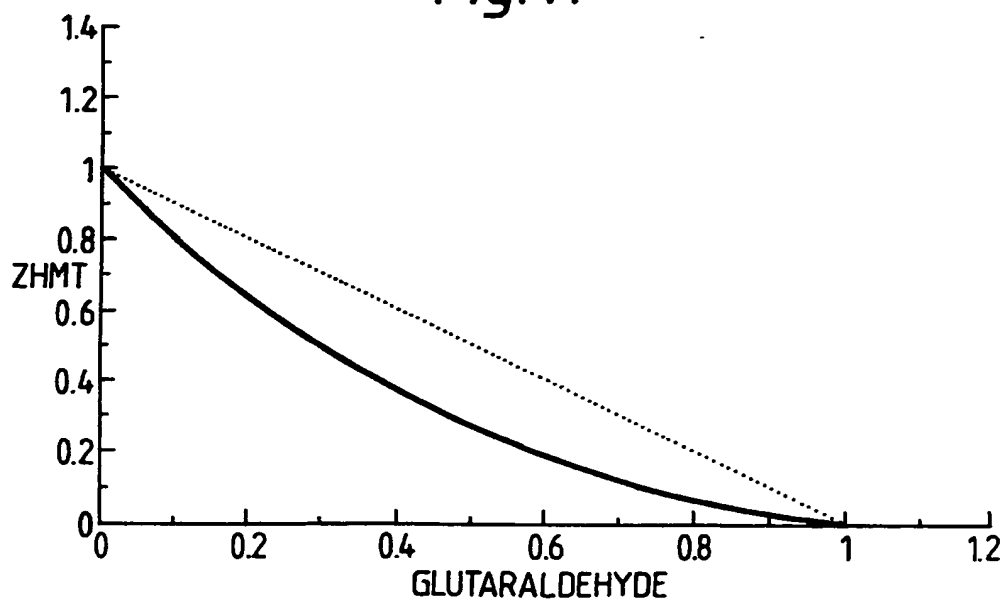
12. A composition as claimed in either claim 10 or claim 11 wherein component (B)(ii) is 2-methylisothiazolin-3-one;  
 5-chloro-2-methylisothiazolin-3-one;  
 4,4-dichloro-2-methylisothiazolin-3-one; 1,2-benzisothiazolin-3-one;  
 4,5-trimethylene-4-isothiazolin-3-one or  
 2-methyl-4,5-trimethylene-4-isothiazoline-3-one.

13. A composition as claimed in either claim 1 or claim 2 wherein component (B)(iii) is formaldehyde or gluteraldehyde.

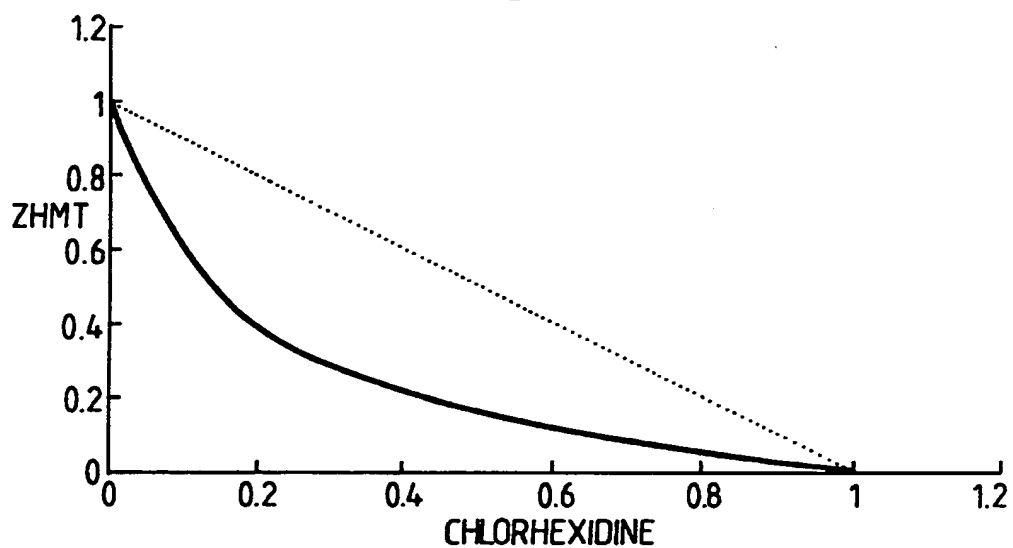
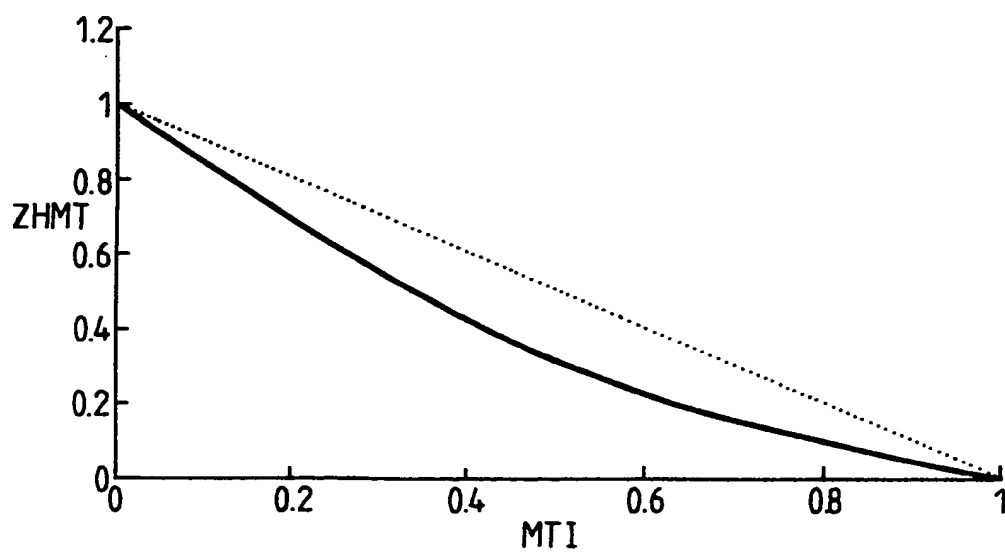
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*Fig.1.**Fig.2.*

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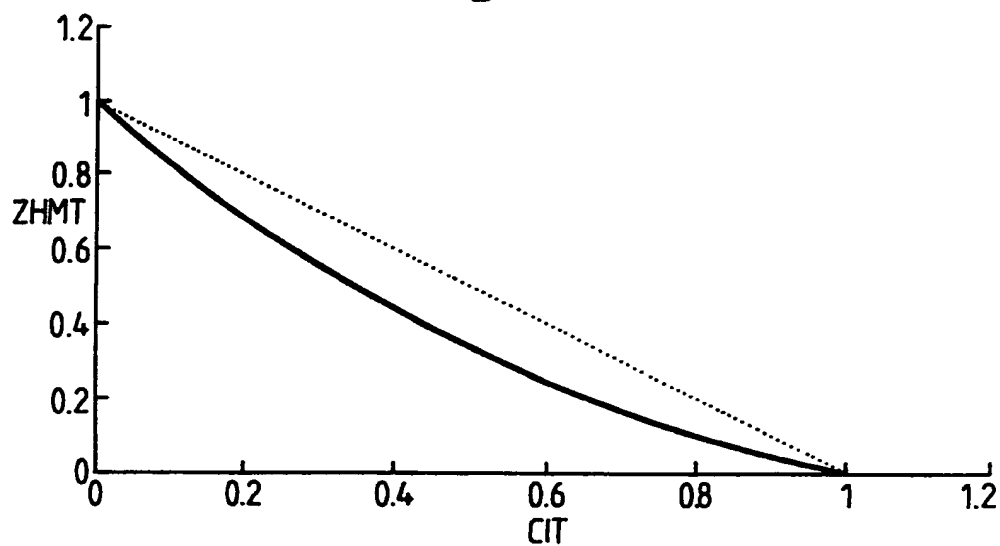
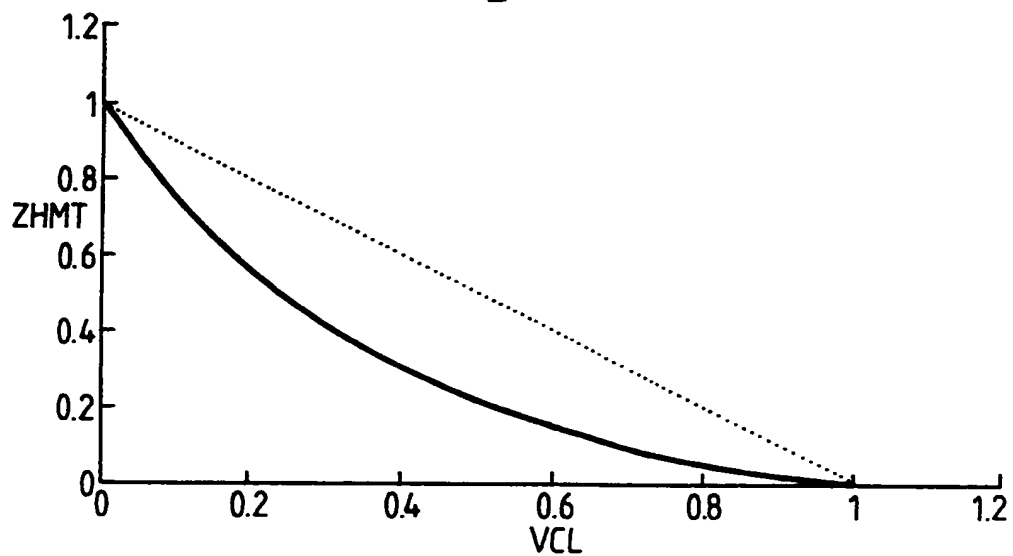
*Fig. 3.**Fig. 4.*

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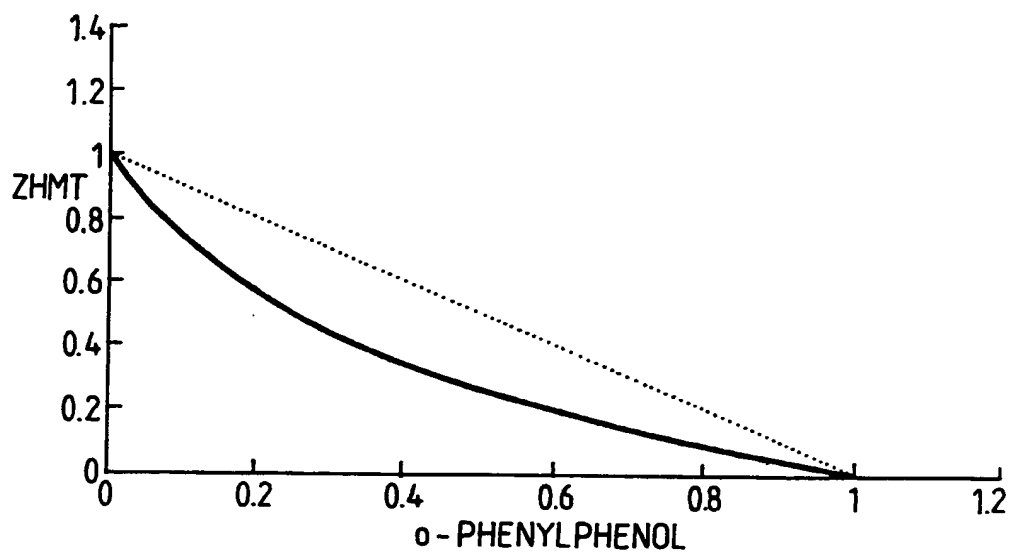
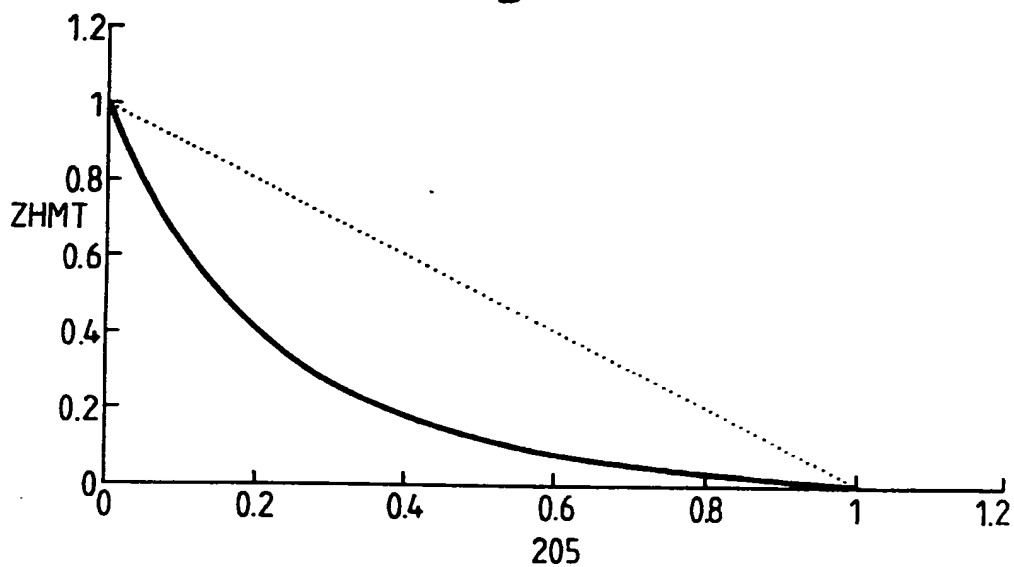
*Fig. 5.**Fig. 6.*



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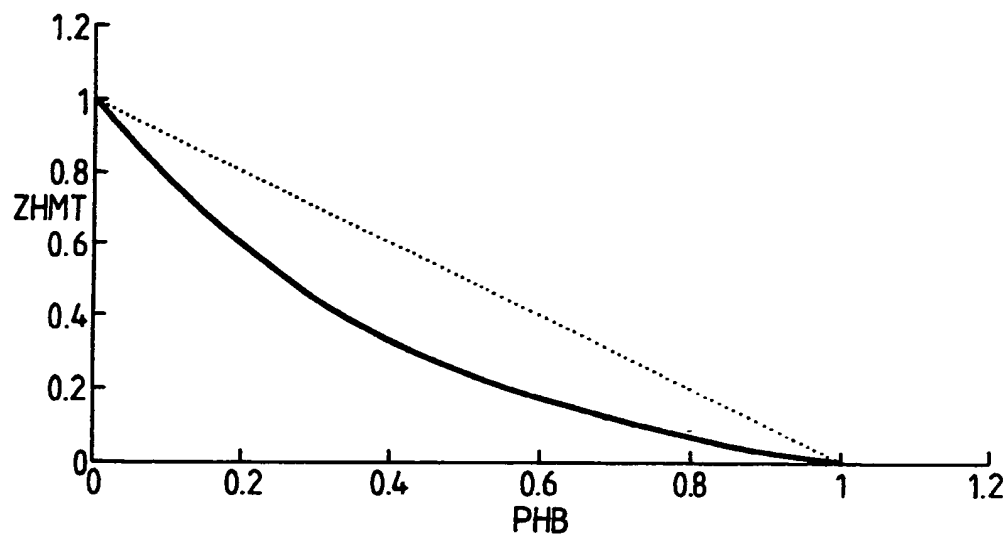
*Fig. 7.**Fig. 8.*

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*Fig. 9.**Fig. 10.*

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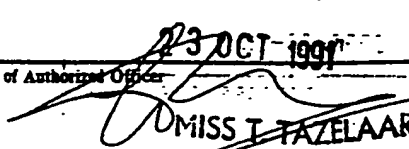
Fig. 11.



# INTERNATIONAL SEARCH REPORT

International Application No.

PCT/EP 91/01293

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) <sup>6</sup>		
According to International Patent Classification (IPC) or to both National Classification and IPC Int.Cl.5      A 01 N 43/78      A 01 N 43/52      A 01 N 43/50 // (A 01 N 43/78, 31:08, 47:44, 33:12, 43:80, 35:02) (A 01 N 43/52, 31:08, 47:44, 33:12, 43:80, 35:02); (A 01 N 43/50, 31:08, 47:44, 33:12, 43:80, 35:02)		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>7</sup>		
Classification System	Classification Symbols	
Int.Cl.5	A 01 N	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched <sup>8</sup>		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT<sup>9</sup></b>		
Category *	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
A	EP,A,0249328 (IMPERIAL CHEMICAL INDUSTRIES PLC) 16 December 1987, (cited in the application) -----	
A	EP,A,0147223 (STERWIN AG) 03 July 1985 -----	
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p><sup>10</sup> Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"A" document member of the same patent family</p> </div> </div>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
07-10-1991	23 OCT 1991	
International Searching Authority	Signature of Authorised Officer	
EUROPEAN PATENT OFFICE	 MISS T. TAZELAAR	

**ANNEX TO THE INTERNATIONAL SEARCH REPORT  
ON INTERNATIONAL PATENT APPLICATION NO.**

EP 9101293

SA 49011

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.  
The members are as contained in the European Patent Office EDP file on 16/10/91  
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0249328	16-12-87	AU-B- 606058	31-01-91
		AU-A- 7294187	26-11-87
		JP-A- 62283963	09-12-87
EP-A- 0147223	03-07-85	AU-A- 3693384	04-07-85
		GB-A- 2151479	24-07-85